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14. ABSTRACT

AMN107 (Novartis Pharmaceuticals) has potent in vitro and in vivo activity against the unmutated and most common mutant forms of Bcr-Abl. Treatment with the histone deacetylase inhibitor LBH589 (Novartis) depletes Bcr-Abl levels. We determined the effects of AMN107 and/or LBH589 in Bcr-Abl–expressing human K562 and LAMA-84 cells, as well as in primary chronic myelogenous leukemia (CML) cells. AMN107 was more potent than imatinib mesylate (IM) in inhibiting Bcr-Abl tyrosine kinase (TK) activity and attenuating p-STAT5, p-AKT, Bcl-xL, and c-Myc levels in K562 and LAMA-84 cells. Cotreatment with LBH589 and AMN107 exerted synergistic apoptotic effects with more attenuation of p-STAT5, p-ERK1/2, c-Myc, and Bcl-xL and increases in p27 and Bim levels. LBH589 attenuated Bcr-Abl levels and induced apoptosis of mouse pro-B BaF3 cells containing ectopic expression of Bcr-Abl or the IM-resistant, point-mutant Bcr-AblT315I and Bcr-AblE255K. Treatment with LBH589 also depleted Bcr-Abl levels and induced apoptosis of IM-resistant primary human CML cells, including those with expression of Bcr-AblT315I. As compared with either agent alone, cotreatment with AMN107 and LBH589 induced more loss of cell viability of primary IM-resistant CML cells. Thus, cotreatment with LBH589 and AMN107 is active against cultured or primary IM-resistant CML cells, including those with expression of Bcr-AblT315I.

15. SUBJECT TERMS
Nilotinib, LBH589, Bcr-Abl, dasatinib, chronic myelogenous leukemia (CML)

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Introduction:

Although anti-Bcr-Abl tyrosine kinase (TK) inhibitor, imatinib, is effective therapy in newly diagnosed patients with CML, resistance to imatinib commonly occurs in patients with accelerated and blastic phase of CML. AMN107 (nilotinib) is more potent anti-Bcr-Abl TK inhibitor, which also inhibits the commonly encountered mutant forms of Bcr-Abl known to confer resistance against imatinib. LBH589 is a potent pan-histone deacetylase (HDAC) inhibitor (HDI), which depletes the levels of Bcr-Abl, by inhibiting the Bcr-Abl chaperone heat shock protein 90 (hsp90). The overall purpose of the studies performed during the previous funding period was to determine the combined effects of LBH589 and nilotinib on unmutated and mutant forms of Bcr-Abl and its downstream signaling proteins. Additionally, these studies were designed to determine the effect of this drug combination on cell growth and apoptosis of Bcr-Abl-containing cultured mouse and human leukemia cells and primary human CML cells.

Body:

AMN107 and LBH589 induce apoptosis of Bcr-Abl–expressing human leukemia cells: We first determined the apoptotic effects of treatment with LBH589 or AMN107 alone on K562 and LAMA-84 cells. Exposure to LBH589 or AMN107 alone induces apoptosis of K562 and LAMA-84 cells in a dose-dependent manner (see Figure 1A and 1B of appended article from Blood). AMN107 was approximately 10-fold more potent than imatinib in inducing apoptosis of K562 and LAMA-84 cells (see Figure 1A of appended article from Blood). Treatment with AMN107 inhibited the levels of tyrosine phosphorylated Bcr-Abl in a dose-dependent manner, without affecting the levels of Bcr-Abl (see Figure 2A of appended article from Blood). AMN107 treatment also inhibited the levels of p-CrkL, suggesting that AMN107 inhibits the TK activity of Bcr-Abl. Treatment with AMN107 attenuated the levels of p-STAT5, as well as lowered the expressions of c-Myc and Bcl-x_L, which are transactivated by STAT5 (see Figure 2B of appended article from Blood). Treatment with AMN107 also inhibited the levels of p-AKT but not AKT, which was associated with induction of p27 levels (see Figure 2B of appended article from Blood).

Co-treatment with LBH589 and AMN107 exerts superior anti–Bcr-Abl activity and synergistically induces apoptosis: As compared with treatment with either agent alone, relatively low concentrations of LBH589 (20 nM) and AMN107 (50 nM) for 24 hours caused more depletion of Bcr-Abl and induced more p27 levels in K562 cells (see Figure 3A of appended article from Blood). In contrast, p21 levels were induced to a similar extent by combined treatment with AMN107 and LBH589, as compared with treatment with LBH589 alone. Combined treatment with LBH589 and AMN107 also caused more attenuation of the levels of p-CrkL, Bcl-xL, and c-Myc but induced more Bim (see Figure 3B of appended article from Blood). Following co-treatment with AMN107 and LBH589, simultaneous induction of Bim and attenuation of Bcl-xL was associated with more PARP cleavage, which is due to increased activity of the effector caspases 3 and 7 during apoptosis. Co-treatment with AMN107 and LBH589 caused significantly more inhibition of colony growth than treatment with either drug alone ($P < .05$) (see Figure 4A of appended article from Blood). Exposure to the combination of AMN107 and LBH589 exerted synergistic apoptotic effect in K562 and LAMA-84 cells, as determined by the median dose-effect isobologram analysis described by Chou and Talalay. For AMN107 and LBH589, the combination index values were less than 1.0 in each cell type (see Figure 4A, 4B and 4C of appended article from Blood). Although AMN107 had no effect (up to

1.0 μM), exposure to 20 and 50 nM LBH589 for 48 hours induced loss of survival of 13.1% and 15.9% of NBMCs (mean of 2 samples with experiments performed in duplicate). Cotreatment with AMN107 did not significantly increase the loss of survival of NBMCs because of exposure to 50 nM LBH589 ($P > .05$).

LBH589 depletes mutant Bcr-Abl levels and induces apoptosis of IM-resistant BaF3 cells expressing Bcr-AblT315I or Bcr-AblE255K: Next, we determined the effect of treatment with LBH589 and/or AMN107 on BaF3 cells with ectopic expression of either the unmutated Bcr-Abl or of the point mutant Bcr-AblE255K or Bcr-AblT315I. AMN107 induced apoptosis of BaF3/Bcr-Abl cells in a dose-dependent manner (see Figure 5A of appended article from Blood). Additionally, co-treatment with AMN107 and LBH589 induced significantly more apoptosis of BaF3/Bcr-Abl cells than either agent alone ($P < .05$) (see Figure 5A of appended article from Blood). Although exposure to IM induced dose-dependent apoptosis of BaF3/Bcr-Abl cells, BaF3/Bcr-AblT315I cells were resistant to IM up to levels as high as 10 μM. In contrast, BaF3/Bcr-AblT315I cells were as sensitive as BaF3/Bcr-Abl cells to apoptosis induced by treatment with LBH589 alone (see Figure 5A and 5B of appended article from Blood). Treatment with 50 nM LBH589 for 48 hours induced apoptosis in approximately 30% of BaF3/Bcr-Abl T315I cells (see Figure 5B of appended article from Blood). Lower levels of LBH589 were less effective. In contrast, BaF3/Bcr-AblT315I cells were resistant to AMN107 levels as high as 2000 nM. Notably, co-treatment with 2000 nM but not 100 nM AMN107 significantly increased LBH589-induced apoptosis of BaF3/Bcr-AblT315I cells ($P < .01$) (see Figure 5B of appended article from Blood). Against BaF3/Bcr-AblE255K cells, although 100 nM AMN107 was ineffective, exposure to 200 and 500 nM AMN107 induced apoptosis of 26.0% and 43.0% of cells, respectively (see Figure 5C of appended article from Blood). Again, co-treatment with AMN107 (500 nM) and LBH589 (50 nM) induced significantly more apoptosis of BaF3/Bcr-AblE255K cells than treatment with either agent alone ($P<.01$), although co-treatment with 100 nM AMN107 was less effective (see Figure 5C of appended article from Blood). Cotreatment with higher concentrations of AMN107 (1.0 or 2.0 μM) also enhanced LBH589-induced apoptosis of BaF3/Bcr-AblE255K. Next, we also correlated the apoptotic effects of AMN107 and/or LBH589 with their effects on the levels of Bcr-Abl in BaF3/Bcr-Abl, BaF3/Bcr-AblE255K, and BaF3/Bcr-AblT315I cells. Treatment with any of the levels of AMN107 tested alone did not lower the levels of Bcr-Abl in any of the 3 cell types (see Figure 6 of appended article from Blood). Exposure to AMN107 also did not affect the levels of p-CrkL or CrkL. In contrast, exposure to 50 nM LBH589 for 24 hours lowered Bcr-Abl levels in all 3 BaF3 transfectants. Notably, as compared with treatment with either agent alone, cotreatment with LBH589 and AMN107 induced more depletion of Bcr-Abl in BaF3/Bcr-Abl cells. Notably, combined treatment with LBH589 and AMN107 caused a more pronounced decline in the levels of Bcr-AblT315I and Bcr-Abl E255K levels in BaF3/Bcr-AblT315I and BaF3/Bcr-AblE255K cells, respectively (see Figure 6 of appended article from Blood). Similar effect was noted on p-CrkL but not CrkL levels.

Cotreatment with AMN107 and LBH589 causes more attenuation of Bcr-Abl and loss of viability of primary, IM-resistant CML cells than either agent alone: We next determined the antileukemia effects of LBH589 and/or AMN107 against primary CML cells isolated from the peripheral blood and/or bone marrow samples from 10 patients who had relapsed with IM-resistant CML-BC. Three of these samples were documented to have the expression of Bcr-AblT315I. In the remaining samples of IM-refractory primary CML cells, the mutational status of Bcr-Abl could not be determined, because of inadequate sample size. In the samples 1 to 7, both AMN107 and LBH589 induced loss of cell viability, which was dose dependent. Additionally, in these samples, co-treatment with LBH589 (20 or 50 nM) and AMN107 induced more loss of cell viability than treatment with either agent alone. Sample 7 was relatively

resistant to lower concentrations of AMN107 but sensitive to LBH589 (see Table 1 of appended article from Blood). In the 3 samples with Bcr-AblT315I mutation, treatment with AMN107 did not augment loss of cell viability, whereas exposure to LBH589 alone for 48 hours markedly inhibited cell viability in a dose-dependent manner (see Table 1 of appended article from Blood). Notably, in these samples (8, 9, and 10), co-treatment with 50 or 100 nM AMN107 did not increase LBH589-induced loss of cell viability (see Table 1 of appended article from Blood). In one sample, although exposure to even 2.0 μM AMN107 was ineffective, co-treatment of 50 nM LBH589 with 2.0 μM AMN107 induced apoptosis of 63.7% of cells, as compared with apoptosis of 42.0% of cells treated with 50 nM LBH589 alone (see Table 1 of appended article from Blood). Western blot analyses of the total cell lysates of one of the samples showed that co-treatment with 50 nM LBH589 and 100 nM AMN107 for 24 hours resulted in more attenuation of Bcr-Abl, p-CrkL, and p-STAT5 than treatment with either agent alone (see Figure 7A of appended article from Blood). In contrast, in sample 9, treatment with even 1000 nM AMN107 alone had little effect on the levels of Bcr-Abl, p-CrkL, and p-STAT5, whereas co-treatment with 50 nM LBH589 and AMN107 markedly depleted the levels of Bcr-AblT315I, as well as of p-CrkL and p-STAT5 (see Figure 7B of appended article from Blood).

Key research accomplishments:

- Combined treatment with the histone deacetylase inhibitor, LBH589, and nilotinib (AMN107) exerts synergistic cytotoxicity against Bcr-Abl positive human acute leukemia cells.

- The underlying molecular mechanisms of the superior anti-leukemia activity of the combination include greater depletion of Bcr-Abl levels and activity, increased levels of Bim and greater attenuation of Bcl-xL.

- LBH589 and nilotinib combination exerts superior activity against imatinib-resistant mutants of Bcr-Abl, i.e., Bcr-AblE255K and Bcr-AblT315I.

Reportable outcomes:

1. Fiskus W, Pranpat M, Bali P, Balasis M, Kumaraswamy S, Boyapalle S, Rocha K, Wu J, Atadja P, Manley P, and Bhalla K. Combined effects of novel tyrosine kinase inhibitor AMN107 and histone deacetylase inhibitor LBH589 against Bcr-Abl expressing human leukemia cells. Blood. 2006;108:645-52

2. W. Fiskus, M. Pranpat, M. Balasis, P. Atadja, P. Manley, F. Giles and K. Bhalla. Combined effects of novel tyrosine kinase inhibitor AMN107 and histone deacetylase inhibitor LBH589 against unmutated or mutant Bcr-Abl-expressing human leukemia cells. Journal of Clinical Oncology, 2006 ASCO Annual Meeting Proceedings Part I. Vol 24, No. 18S (June 20 Supplement), 2006: 6592

3. H. M. Kantarjian, N. Gattermann, S. G. O'Brien, K. Bhalla, A. Hochhaus, F. Cervantes, L. Alland, O. Ottmann, F. Giles and P. Le Coutre. A phase II study of AMN107, a novel inhibitor of Bcr-Abl, administered to imatinib resistant and intolerant patients (pts) with chronic myelogenous leukemia (CML) in chronic phase (CP). Journal of Clinical Oncology, 2006 ASCO Annual Meeting Proceedings Part I. Vol 24, No. 18S (June 20 Supplement), 2006: 6534

4. O. Ottmann, K. Bhalla, H. Kantarjian, A. Hochhaus, D. Jones, K. Dawson, K. Rose, L. Alland, M. Dugan, S. Lilleburg and F. Giles. Bcr-Abl mutations in imatinib-resistant CML and Ph+ALL patients (pts) enrolled in a phase I study of AMN107. Journal of Clinical Oncology, 2006 ASCO Annual Meeting Proceedings Part I. Vol 24, No. 18S (June 20 Supplement), 2006: 6527

5. C. Tanaka, T. Smith, H. Kantarjian, F. Giles, O. Ottmann, K. Bhalla, K. Grouss, V. Sethuraman, K. Thomas and H. Schran. Clinical pharmacokinetics (PK) of AMN107, a novel inhibitor of Bcr-Abl, in healthy subjects and patients with imatinib resistant or intolerant chronic myelogenous leukemia (CML) or relapsed/refractory Ph+ acute lymphocytic leukemia (Ph+ALL). Journal of Clinical Oncology, 2006 ASCO Annual Meeting Proceedings Part I. Vol 24, No. 18S (June 20 Supplement), 2006: 3095

6. K. N. Bhalla, P. Bali, M. Balasis, W. Fiskus, M. Pranpat, K. Rocha, R. M. Rao, S. Kumaraswamy and P. Atadja. Inhibition of histone deacetylase (HDAC) 6 sensitizes human leukemia and breast cancer cells to antagonists of heat shock protein (hsp) 90 and/or bortezomib (BZ). Journal of Clinical Oncology, 2006 ASCO Annual Meeting Proceedings Part I. Vol 24, No. 18S (June 20 Supplement), 2006: 13039

7. T. Fischer, A. Patnaik, K. Bhalla, J. Beck, J. Morganroth, G. H. Laird, S. Sharma, J. W. Scott, M. Dugan and F. Giles. Results of cardiac monitoring during phase I trials of a novel histone deacetylase (HDAC) inhibitor LBH589 in patients with advanced solid tumors and hematologic malignancies. Journal of Clinical Oncology, 2005 ASCO Annual Meeting Proceedings. Vol 23, No. 16S, Part I of II (June 1 Supplement), 2005: 3106

8. P. Bali, F. Guo, W. Fiskus, M. Pranpat, C. Sigua and K. Bhalla. Mechanisms underlying hydroxamic acid analogue (HA) histone deacetylase (HDAC) inhibitors (HDIs)-induced apoptosis: New role of HDAC6 inhibition, acetylation and inhibition of hsp90 and depletion of pro-growth and pro-survival oncoproteins. American Association for Cancer Research 96th Annual Meeting, Anaheim/Orange County, CA, April 16-20, 2005

Conclusion:

The results of our studies have demonstrated that cotreatment with LBH589 and nilotinib exerts superior anti-Bcr-Abl activity and synergistically induces apoptosis of Bcr-Abl-positive human leukemia cells. While nilotinib did not deplete levels of Bcr-Abl, it significantly reduced TK activity of unmutated Bcr-Abl, as well as induced apoptosis of unmutated Bcr-Abl or Bcr-AblE255K expressing mouse and human leukemia cells. LBH589 depleted the levels of Bcr-Abl in BaF3/Bcr-Abl, BaF3/Bcr-AblE255K and BaF3/Bcr-AblT315I cells. Cotreatment with nilotinib and LBH589 caused more attenuation of Bcr-Abl and greater loss of cell viability of primary imatinib-resistant CML cells than treatment with either agent alone. This was associated with significantly greater depletion of the levels of unmutated Bcr-Abl or Bcr-AblT315I, as well as of p-CrkL and p-STAT5. These findings strongly support the rationale to test the activity and efficacy of the combination of LBH589 and nilotinib against Bcr-Abl positive leukemia sensitive or resistant to imatinib with or without mutant forms of Bcr-Abl.

References:

1. Ren R. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. Nat Rev Cancer. 2005;5:172-183.

2. Sawyers CL. Opportunities and challenges in the development of kinase inhibitor therapy for cancer. Genes Dev. 2003;17:2998-3010.

3. Hoover RR, Gerlach MJ, Koh EY, Daley GQ. Cooperative and redundant effects of STAT5 and Ras signaling in BCR/ABL transformed hematopoietic cells. Oncogene. 2001;20:5826-5835.

4. Rascle A, Johnston JA, Amati B. Deacetylase activity is required for recruitment of the basal transcription machinery and transactivation by
STAT5. Mol Cell Biol. 2003;23:4162-4173.

5. Skorski T, Bellacosa A, Nieborowska-Skorska M, et al. Transformation of hematopoietic cells by BCR/ABL requires activation of a PI-3k/Akt-dependent
pathway. EMBO J. 1997;16:6151-6161.

6. Kuwana T, Bouchier-Hayes L, Chipuk JE, et al. BH3 domains of BH3-only proteins differentially regulate Bax-mediated mitochondrial membrane
permeabilization both directly and indirectly. Mol Cell. 2005;17:525-535.

7. Aichberger KJ, Mayerhofer M, Krauth MT, et al. Low-level expression of proapoptotic Bcl-2-interacting mediator in leukemic cells in patients with chronic myeloid leukemia: role of BCR/ABL, characterization of underlying signaling pathways, and reexpression by novel pharmacologic compounds. Cancer Res. 2005;65:9436-9444.

8. Shah NP, Nicoll JM, Nagar B, et al. Multiple Bcr-Abl kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell. 2002;2:117-125.

9. Hochhaus A, La Rosee P. Imatinib therapy in chronic myelogenous leukemia: strategies to avoid and overcome resistance. Leukemia. 2004;18:1321-1331.

10. Michor F, Hughes TP, Iwasa Y, et al. Dynamics of chronic myeloid leukaemia. Nature. 2005;435:1267-1270.

11. Golemovic M, Verstovsek S, Giles F, et al. AMN107, a novel aminopyrimidine inhibitor of Bcr-Abl, has in vitro activity against imatinib-resistant chronic myeloid leukemia. Clin Cancer Res. 2005;11:4941-4947.

12. Weisberg E, Manley PW, Breitenstein W, et al. Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. Cancer Cell. 2005;7:129-141.

13. O'Hare T, Walters DK, Stoffregen EP, et al. In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib resistant
Abl kinase domain mutants. Cancer Res. 2005;65:4500-4505.

14. Bhalla KN. Epigenetic and chromatin modifiers as targeted therapy of hematologic malignancies. J Clin Oncol. 2005;23:3971-3993.

15. Lindemann RK, Gabrielli B, Johnstone RW. Histone-deacetylase inhibitors for the treatment of cancer. Cell Cycle. 2004;3:779-788.

16. Guo F, Sigua C, Tao J, et al. Cotreatment with histone deacetylase inhibitor LAQ824 enhances Apo-2L/tumor necrosis factor-related apoptosis inducing ligand-induced death inducing signaling complex activity and apoptosis of human acute leukemia cells. Cancer Res. 2004;64:2580-2589.

17. Nimmanapalli R, Fuino L, Stobaugh C, Richon V, Bhalla K. Cotreatment with the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) enhances imatinib-induced apoptosis of Bcr-Abl-positive human acute leukemia cells. Blood. 2003;101:3236-3239.

18. Nimmanapalli R, Fuino L, Bali P, et al. Histone deacetylase inhibitor LAQ824 both lowers expression and promotes proteasomal degradation of Bcr-Abl and induces apoptosis of Imatinib Mesylate-sensitive or -refractory chronic myelogenous leukemia-blast crisis cells. Cancer Res. 2003;63:5126-5135.

19. George P, Bali P, Annavarapu S, et al. Combination of the histone deacetylase inhibitor LBH589 and the hsp90 inhibitor 17-AAG is highly active against human CML-BC cells and AML cells with activating mutation of FLT-3. Blood. 2005;105:1768-1776.

20. Bali P, Pranpat M, Bradner J, et al. Inhibition of histone deacetylase 6 acetylates and disrupts the chaperone function of heat shock protein 90: a novel basis for antileukemia activity of histone deacetylase inhibitors. J Biol Chem. 2005;280:26729-26734.

21. Bagatell R, Whitesell L. Altered Hsp90 function in cancer: a unique therapeutic opportunity. Mol Cancer Ther. 2004;3:1021-1030.

22. Gorre ME, Ellwood-Yen K, Chiosis G, Rosen N, Sawyers CL. BCR-ABL point mutants isolated from patients with imatinib mesylate-resistant chronic myeloid leukemia remain sensitive to inhibitors of the BCR-ABL chaperone heat shock protein 90. Blood. 2002;100:3041-3044.

23. Shah NP, Tran C, Lee FL, Chen P, Norris D, Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. Science. 2004;305:399-401.

24. Andreu EJ, Lledo E, Poch E, et al. BCR-ABL induces the expression of Skp2 through the PI3K pathway to promote p27Kip1 degradation and proliferation of chronic myelogenous leukemia cells. Cancer Res. 2005;65:3264-3272.

25. Nimmanapalli R, O'Bryan E, Bhalla K. Geldanamycin and its analogue 17-allylamino-17-demothoxygeldanamycin (17-AAG) lowers Bcr-Abl level and induces apoptosis and differentiation of Bcr-Abl positive human leukemic blasts. Cancer Res. 2001;61:1799-1804.

26. Nimmanapalli R, O'Bryan E, Kuhn D, Yamaguchi H, Wang H-G, Bhalla, K. Regulation of 17-AAG induced apoptosis: role of Bcl-2, Bcl-xL, and Bax downstream of 17-AAG-mediated down-regulation of Akt, Raf-1, and Src kinases. Blood. 2003;102:269-275.

27. Gambacorti-Passerini C, Gasser M, Ahmed S, Assouline S, Scapozza L. Abl inhibitor BMS354825 binding mode in Abelson kinase revealed by molecular docking studies. Leukemia. 2005;19:1267-1269.

28. Burgess MR, Skaggs BJ, Shah NP, Lee FY, Sawyers CL. Comparative analysis of two clinically active BCR-ABL kinase inhibitors reveals the role of conformation-specific binding in resistance. Proc Natl Acad Sci U S A. 2005;102:3395-3400.